Persistence of Mitochondrial Toxicity in Hearts of Female B6C3F1 Mice Exposed \textit{In Utero} to 3'-Azido-3'-Deoxythymidine

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Abstract

Cardiac toxicity has been associated with HIV infection and exposure to nucleoside reverse transcriptase inhibitors (NRTIs), but the role of the latter in the development of cardiac disease of HIV-infected patients is uncertain. To investigate the cardiotoxicity of transplacentally administered zidovudine (AZT) or AZT plus lamivudine (3TC) in the absence of HIV infection, we evaluated several biomarkers of cardiac mitochondrial structure and cardiac structure and function in a B6C3F1 mouse model. \textit{In utero} exposure to AZT-3TC resulted in ultrastructural pathology, loss of mitochondria, and altered echocardiographic measurements in newborn mice. Cardiac pathology and dysfunction persisted into the adult life of female mice exposed \textit{in utero} to AZT, as evidenced by significant dose-dependent heart enlargement, clusters of atypical mitochondria and myofibril alterations, significantly increased cytochrome \textit{c} oxidase activity, and significantly higher numbers of mutations in mitochondrial tRNA genes compared with unexposed controls at 18 to 24 mo of age. These data led to the hypothesis that the long-term pathology of perinatal exposure to these NRTIs is related to persistent mitochondrial DNA mutations in cardiac tissue; that is, the primary damage during drug treatment is mutational (as opposed to affecting polymerase \textit{γ} and/or other mitochondrial elements) and leads over time to delayed, progressive cardiotoxicity.

Key Words: Mitochondria; AZT; 3TC; cardiomyopathy; DGGE; mitochondrial DNA mutations.

Introduction

The use of nucleoside reverse transcriptase inhibitors (NRTIs) to control the progression of HIV-1 infection and to reduce the incidence of maternal–fetal viral transmission has greatly prolonged the lives of affected individuals and has dramatically reduced vertical transmission rates. Concomitant with these advantages are risks for deleterious toxicities, especially to mitochondrial-rich tissues such as the heart. Long-term use of NRTIs probably reduces their benefits in some patients, as evidenced by the development of cardiomyopathy in a significant portion of
HIV-infected patients receiving highly active antiretroviral therapy. In addition, the use of NRTIs during pregnancy to control vertical transmission of the virus may pose unique risks during the growth and lifetime of uninfected children born to HIV-infected mothers.

Several studies document the incidence of cardiac disease in HIV-infected adults. Jacob et al. (1) found that 26/173 (15%) of adult HIV-infected patients had cardiac abnormalities as evidenced by changes in ventricular size, function, or both. Roy et al. (2) reported that 24% of inner-city hospital patients infected with HIV had cardiomyopathy. In another study, symptomatic heart failure associated with the presence of a dilated cardiomyopathy, or left or right ventricular dysfunction, was found in up to 5% of HIV-positive patients (3).

The pathogenesis of cardiac disease in HIV-infected patients is uncertain. Investigators have suggested that mitochondrial dysfunction is a critical element in the development of AIDS or NRTI-related cardiomyopathy (4,5), although the precise role that each component plays is unclear. This mitochondrial dysfunction appears to arise from several different phenomena that may occur simultaneously in AIDS and/or following exposure to NRTIs. These phenomena include (1) mitochondrial DNA (mtDNA) depletion (resulting from drug-related interference with DNA polymerase γ, inhibition of mtDNA replication and inhibition of DNA polymerase γ exonuclease activity (6,7); (2) increased mitochondrial oxidative stress, resulting from HIV infection, NRTI exposure, or both; (3) energy depletion as a result of 1 and 2 (8); and (4) induction of mutations into mtDNA and consequent respiratory chain dysfunction (9). The relative contribution of the virus vs antiretroviral drugs to mitochondrial compromise observed in HIV-infected patients can be difficult to distinguish; however, in experimental systems treated with NRTIs in the absence of viral infection, the phenomena leading to mitochondrial damage/dysfunction can be investigated and attributed to the drug(s) alone (9).

Compared with the large body of evidence documenting an association between antiretroviral therapy, mitochondrial abnormalities, and cardiomyopathy in HIV-infected adults (9), few published data suggest a potential link between childhood treatment of HIV and mitochondrial dysfunction/cardiac disease. Domanski et al. (10) performed a retrospective analysis of 137 HIV-infected children who had been treated with no antiretroviral therapy, with zidovudine (3'-azido-3'-deoxythymidine, AZT), with didanosine, or with both drugs. A highly significant relationship between length of AZT treatment (in days) and decreased average fractional shortening was found in AZT-treated children, even after the echocardiographic results were corrected for the severity of HIV disease. No such relationship was found for didanosine. Dagan et al. (9) reported the results of a prospective study of treated HIV-infected pediatric patients over a 2-yr period and found a 10–15% cumulative incidence of left ventricular dysfunction. These results support a relationship between cumulative AZT dose, length of AZT therapy, and risk of cardiac dysfunction.

The work reported here used an experimental animal model, lacking the complications of HIV disease, to elucidate the mechanisms underlying the risk for persistent mitochondrial toxicity and cardiomyopathy following in utero exposure to AZT. During recently completed 2-yr transplacental carcinogenicity studies of AZT in B6C3F1 mice and F344 rats (11), exposed offspring failed to grow at the rate of unexposed animals and developed a treatment-related complex of noncancerous lesions, including a dose-dependent cardiomyopathy that was most pronounced in female mice. Given these findings, hearts were collected from groups of adult animals to assess the relationships between in utero AZT exposure, mitochondrial damage/dysfunction, and the observed cardiomyopathy. The results of subsequent pilot studies, presented here, demonstrate significant treatment-related differences in ultrastructural pathology of cardiac mitochondria, cytochrome c oxidase (COX) staining of heart tissue, and frequency of small-scale mtDNA mutations in hearts from middle-aged (18 mo) control mice vs mice exposed to AZT during the last approx 40% of gestation. Also described are electron microscopy (EM) findings in hearts of newborn mice, and echocardiographic measurements in mice at as early an age as possible (7 wk), after transplacental exposure to AZT plus lamivudine (2'-deoxy-3'-thiacytidine, 3TC).

Materials and Methods

Chemicals, Animals, and Animal Exposures

AZT and 3TC were purchased from Sigma (St. Louis, MO) and Byron Chemical (Long Island City,